# miRNA

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REVIEW Open Access

# Dysregulation of MicroRNAs in cancer

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## MicroRNAs in Mutagenesis, Genomic Instability and DNA Repair

Dan-Avi Landau[1] and Frank J. Slack[2]

Upsala Journal of Medical Sciences. 2012; 117: 202–216

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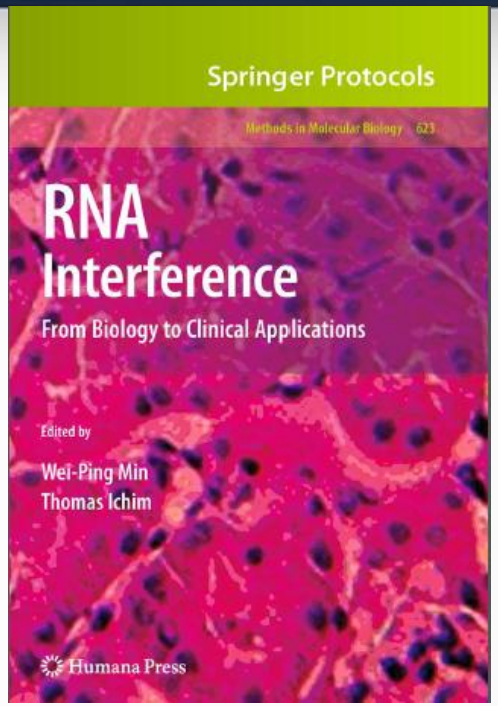


REVIEW ARTICLE

## MicroRNA in cancer: New hopes for antineoplastic chemotherapy

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REVIEW

## DNA methylation and microRNAs in cancer

Xiang-Quan Li, Yuan-Yuan Guo, Wei De

- MicroRNAs (miRNAs) are small noncoding RNAs which enhance the cleavage or translational repression of specific mRNA with recognition site(s) in the 3’-untranslated region (3’UTR).

- The biogenesis of miRNA is controlled by two RNase-dependent processing steps that converts a long primary transcript into a mature ~20 nt miRNA.

- The mature miRNA are released and then loaded onto the miRNA-induced silencing complex (miRISC), which acts as a guiding strand to recognize specific mRNA targets.

# Canonical generation of pre-miRNAs

- miRNA genes are transcribed in the nucleus mainly by RNA polymerase (pol) II into stem-loop structured primary miRNAs (pri-miRNAs).

- Harboring a 5’ m7G cap and a 3’ poly(A) tail, these pri-miRNAs are then trimmed into ~60 to 70-nt miRNA precursors (pre-miRNAs) by the nuclear ribonuclease (RNase) III Drosha, acting in concert with the DiGeorge syndrome critical region 8 (DGCR8) protein within the microprocessor complex.

# Noncanonical generation of pre-miRNAs

- in which certain debranched small introns mimic the structural features of pre-miRNAs to enter the miRNA-processing pathway without Drosha-mediated cleavage, named “mirtrons”, has been described initially in *Drosophila,* but are also present in mammals. To date, no viral “mirtrons” have been reported.

- The canonical and noncanonical pre-miRNAs are subsequently exported to the cytoplasm via Exportin-5, and the base of their stem is recognized by the PAZ domain of Dicer.

- Acting as an intramolecular dimer, Dicer RNase IIIa and IIIb domains cleave the stem at the base of the loop to generate a miRNA:miRNA* duplex.

- The transactivating response RNA-binding protein (TRBP) has been shown to operate with Dicer within a pre-miRNA processing complex, although their precise mechanistic interaction remains elusive. Following a strand selection and separation step, which is based on the thermodynamic stability of the RNA duplex, the miRNA strand (~21 to 24-nt) with the least stable 5’ end pairing (called the guide strand) is incorporated into effector miRNA-containing ribonucleoprotein (miRNP) complexes containing Argonaute 2 (Ago2), TRBP and Dicer, guiding them toward specific messenger RNAs (mRNAs).

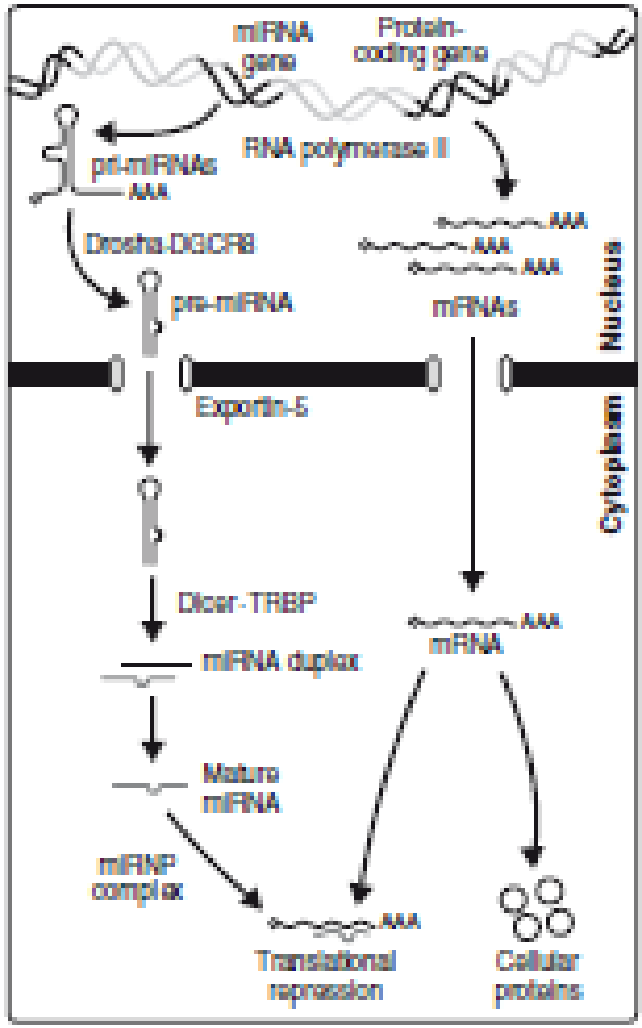


Fig. 1. Schematic representation of the miRNA-guided RNA silencing pathway in mammalian cells. miRNA genes are transcribed mainly by RNA polymerase II into primary miRNAs (pri-miRNAs). These RNA species are then trimmed into miRNA precursors (pre-miRNAs) in the nucleus by the microprocessor complex, which is composed of the ribonuclease Drosha and its cofactor DGCR8. Pre-miRNAs are then exported by Exportin-5 to the cytoplasm, where they are cleaved by the pre-miRNA processing complex formed of Dicer and TRBP, to generate miRNA:miRNA* duplexes. Following a strand selection and separation step, the mature miRNA is incorporated into an Ago2-containing miRNA ribonucleoprotein complex (miRNP) (or effector complex) to mediate recognition and translational repression of specific cellular mRNAs

- Cellular miRNAs have been shown to control various processes such as cell proliferation, apoptosis and hematopoietic cell differentiation. As for viral miRNAs, they can regulate expression of viral as well as host proteins by directing repression or cleavage of mRNA transcripts, thereby inhibiting key cellular processes involved in the response to viral infection.

**Table 1**
**MicroRNAs and noncoding RNAs expressed by human viruses**

| Virus family | Virus name | miRNAs/ncRNAs Predicted | Validated | Identification of validated miRNAs/ncRNAs | References |
|---|---|---|---|---|---|
| Herpesvirus | Herpes simplex virus type 1 (HSV-1, HHV-1) | (mi) 24 | (mi) 8 | hsv-miR-H1, miR-H2-3p, miR-H4-3p, miR-H2-5p, miR-H3 , miR-H5, miR-H6, miR-I | (51, 52, 61, 64) |
| | | 0 | (nc) 2 | –38 and –110 nt from the LAT transcript | (55) |
| | Herpes simplex virus type 2 (HSV-2, HHV-2) | (mi) 10 | 0 | – | (51, 55) |
| | Varicella zoster virus (VZV, HHV-3) | 0 | 0 | – | – |
| | Epstein–Barr virus (EBV, HHV-4) | (mi) 7 | (mi) 23 | miR-BHRF1-1 to BHRF1-3, miR-BART-1 to miR-BART-20 | (51, 52, 66) (70, 73) |
| | | | (nc) 2 | EBER1, EBER2 | (8, 125, 126) |
| | Human cytomegalovirus (HCMV, HHV-5) | (mi) 11 | (mi) 11 | miR-UL22A, miR-UL36, mir-UL70, miR-UL112, miR-UL148D, miR-US4, miR-US5-1,miR-US5-2, miR-US25-1, miR-US25-2, miR-US33 | (50, 80, 81) |
| | | | (nc) 1 | β2.7 | (127) |
| | Roseolo virus (HHV-6) | 0 | 0 | – | – |
| | HHV-7 | 0 | 0 | – | – |
| | Kaposi's sarcoma-associated herpesvirus (KSHV, HHV-8) | (mi) 8 | (mi) 12 | miR-K12-1 to miR-K12-12 | (51, 66, 91, 92) |

**Table 1**
**MicroRNAs and noncoding RNAs expressed by human viruses**

| Virus family | Virus name | miRNAs/ncRNAs Predicted | Validated | Identification of validated miRNAs/ncRNAs | References |
|---|---|---|---|---|---|
| Polyomavirus | Simian virus 40 (SV40) | (mi) 1 | (mi) 2 | sv40-miR-S1-5p, sv40-miR-S1-3p | (51,101) |
| | Simian virus 12 (SV12) | ND | (mi) 2 | unnamed | (102) |
| | Jamestown Canyon virus (JCK) | (mi) 1 | (mi) 2 | unnamed | (103) |
| | BKV | (mi) 1 | (mi) 2 | unnamed | (103) |
| Adenovirus | Adenovirus type 2 and 5 | 0<br>(mi) 1 | (nc) 2<br>(mi) 3 | VAI, VAII<br>mivaRI-137, mivaRI-138 (or 3'svaRNA), mivaRII-138 | (12, 104)<br>(47, 104–107) |
| Retrovirus | Human immunodeficiency virus type 1 | 0 | (mi) 3 | miR-N367, miR-TAR-5p, miR-TAR-3p | (48, 113, 116) |
| | Human immunodeficiency virus type 2 | (mi) 2 | 0 | – | (118) |
| | HTLV-1 | 0 | 0 | – | (119) |

# miRNAs in cancer

- Genome-wide miRNA expression profiling analyses have reported a general dysregulation of miRNA expression in all tumors. To date, over 1000 miRNAs have been reported in humans (miRbase:1527 at November 2011), and hundreds of them map to chromosomic regions that are known to be altered in human cancer, such as loss of heterozygosity regions (LOH) (e.g. miR-15a/16-1), amplified regions (e.g. miR-17–92 cluster, miR-155), and breakpoint regions and fragile sites (FRA) (e.g. let-7 family members).

- Published studies have defined that elevated expression of some miRNAs (oncogenes) as well as down-regulation of others (tumor-suppressors) accompanies carcinogenesis and correlates with the development of cancer-associated phenotypes.

# Emerging roles of miRNAs in cancer

1. Tumorigenesis
2. Tumor metastasis
3. Drug resistance

# Emerging roles of miRNAs in cancer tumorigenesis

1. Let-7 is the most studied miRNA both in development and cancer. The human let-7 family comprises 12 closely related members of miRNA (let-7-a-1, a-2, a-3, b, c, d, e, f-1, f-2, g, i and miR-98).

It was reported that let-7 is downregulated in lung cancer and is associated with elevated RAS expression. They further showed that let-7 is complementary to multiple sites in the 3'UTR of the human RAS genes, allowing let-7 to suppress the expression of K-RAS and N-RAS. The tumor suppressive roles of let-7 are further strengthened by its antagonistic roles toward the expression of multiple oncogenes including RAS, MYC, and other cell cycle regulators in a variety of human cancer tissues.

2. MiRNAs derived from miR-17-92 cluster, which contains seven homologous miRNAs, including miR-17-3p, miR-17-5p, miR-18a, miR-20a, miR-19a, miR-19b-1, and miR-92a-1, have been identified as oncogenic miRNAs. These miRNAs target multiple genes involved in proapoptotic pathways, reflecting their oncogenic activities.
3. MiR-21 has been shown to be overexpressed in a wide variety of cancers, including malignant human glioblastoma tumor tissues. Knockdown of miR-21 induced activation of caspases and resulted in apoptosis in glioblastoma cells.

# The roles of miRNAs in tumor metastasis

- MiR-10b is the most studied miRNA with metastasis-promoting effect and is directly regulated by Twist1, an oncoprotein facilitating epithelial-mesenchymal transition (EMT). Expression of miR-10b is markedly elevated and maintains the invasiveness of metastatic human breast cancer cells. Overexpression of miR-10b in non-metastatic breast cancer cells results in enhanced invasiveness and distant metastasis.

# Functions of miRNAs in drug resistance

- MiR-519c was first found to increase drug sensitivity of colon cancer cells by regulating ABCG2 and was later shown to suppress the expression of HIF-1α which consequently attenuates tumor angiogenesis

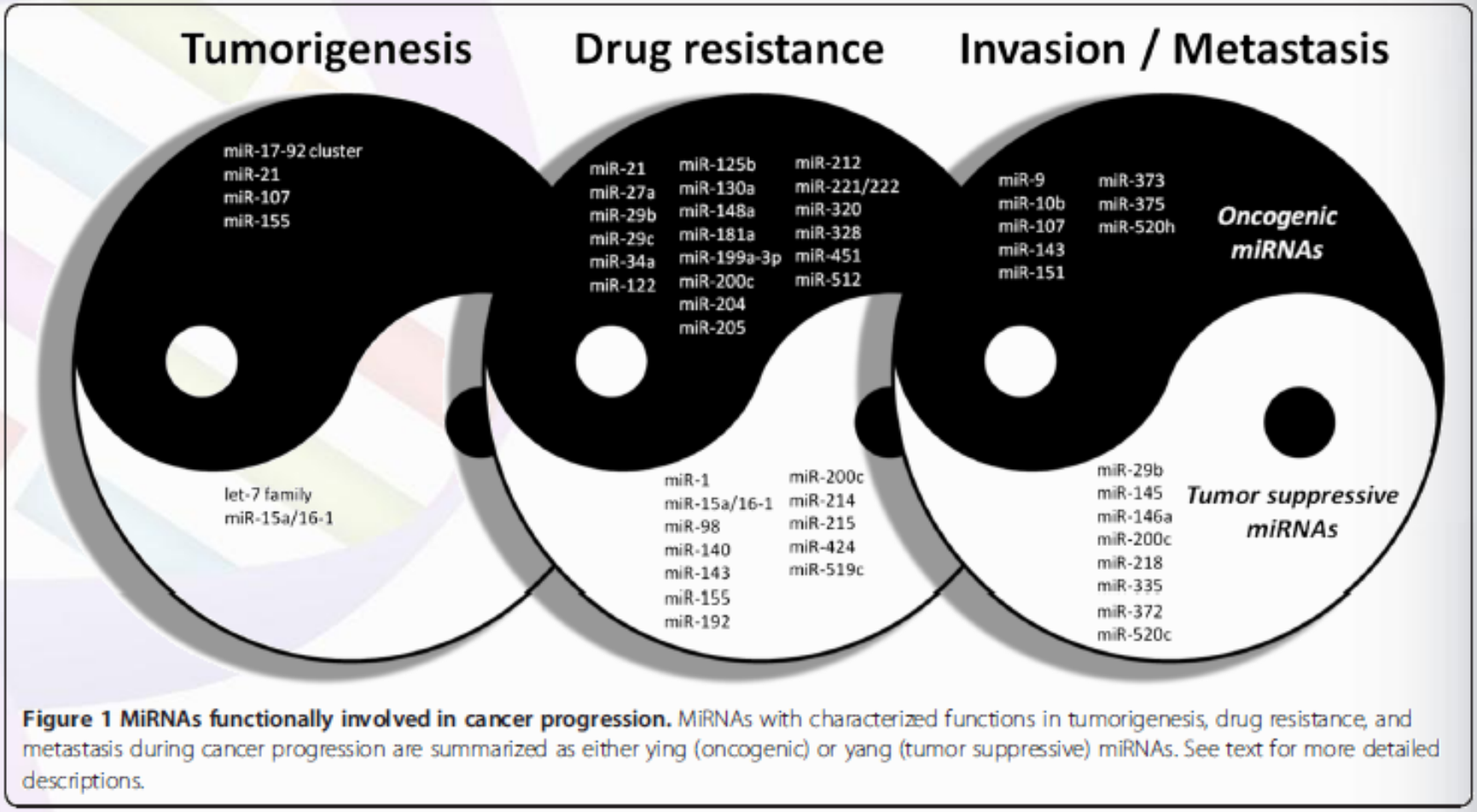


**Figure 1 MiRNAs functionally involved in cancer progression.** MiRNAs with characterized functions in tumorigenesis, drug resistance, and metastasis during cancer progression are summarized as either ying (oncogenic) or yang (tumor suppressive) miRNAs. See text for more detailed descriptions.

# Mechanisms of dysregulation of miRNAs in cancer

# 1. Genomic abnormalities

- Like protein-coding genes, more than half of miRNA genes in human cancers are located in chromosomal regions that frequently exhibit amplification, deletion, or translocation

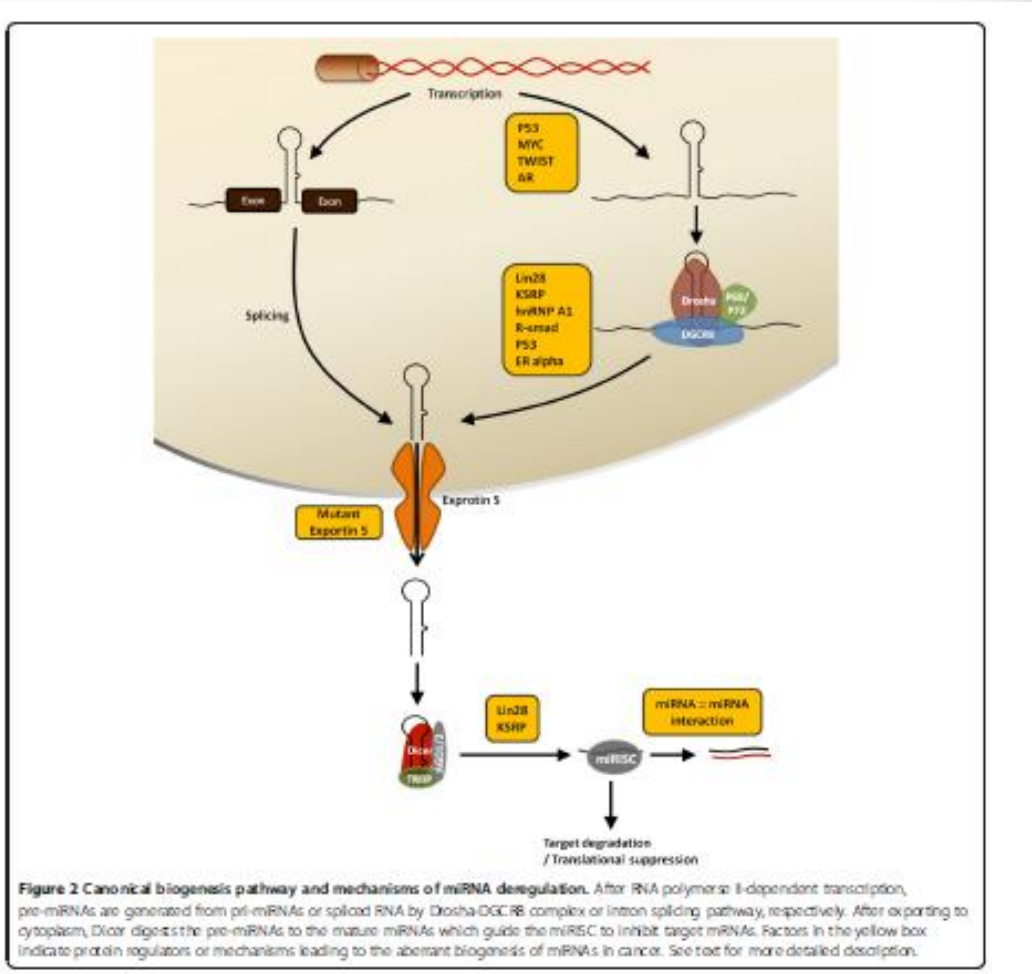


**Figure 2 Canonical biogenesis pathway and mechanisms of miRNA deregulation.** After RNA polymerase II-dependent transcription, pre-miRNAs are generated from pri-miRNAs or spliced RNA by Drosha-DGCR8 complex or intron splicing pathway, respectively. After exporting to cytoplasm, Dicer digests the pre-miRNAs to the mature miRNAs which guide the miRISC to inhibit target mRNAs. Factors in the yellow box indicate protein regulators or mechanisms leading to the aberrant biogenesis of miRNAs in cancer. See text for more detailed description.

# Examples

- The region 13q14 of the chromosome where miR-15 and miR-16 are located and frequently deleted in B cell chronic lymphocytic leukemias (B-CLL), resulting in the loss or downregulated expression of miR-15 and miR-16.
- Deletion of miR-17-92 cluster exists in melanomas, ovarian, and breast cancers.
- The oncogenic miR-155 was found to be upregulated along with its host gene, BIC, in Burkitt's lymphoma patients.

- These studies provide an important connection between the expression of miRNAs and genomic deletion/amplification in cancer.

# 2. CpG methylation and histone modification

- Transcriptional silencing of tumor suppressor genes by CpG island promoter hypermethylation is a common hallmark of cancer.
- Similar phenomenon has been identified in miRNA regulation in which Saito et al. showed that a subset of miRNAs is upregulated by treatment of inhibitors specific for DNA methylation (5-aza-20-deoxycytidine) or histone deacetylase (4-phenylbutyric acid) in cancer cells.

# Examples

- miR-127 is downregulated in human cancers. MiR-127 is embedded in a CpG island and dramatically upregulated through its own promoter, suggesting that DNA methylation or histone modification at this promoter region hinders the expression of miR-127 in cancer cells. The downstream target of miR-127, Bcl-6, is also consistently repressed after the treatments.

- It was investigated the profile of miRNA expression in cells lacking DNA methyltransferases and found that miRNA-124a is downregulated by CpG island hypermethylation. This epigenetic silencing subsequently activates CDK6 and induces Rb phosphorylation.

- One of the let-7 genes, Let-7a-3, is also located within the CpG islands. It was found that let-7a-3 gene is hypermethylated in ovarian cancer and hypermethylated let-7a-3 is associated with downregulation of IGFII expression and poor prognosis in ovarian cancer patients, suggesting that let-7 expression may target IGF-II.

# 3. Transcriptional regulation

- miRNA expression is also regulated by transcription factors.
- p53 is a fundamental tumor suppressor which transcriptionally regulates hundreds of protein coding genes. In 2007, three studies that published at the same time uncovered the subsets of miRNA regulated by p53. They analyzed the profiles of p53-dependent miRNA expression and found that a family of these miRNAs, miR-34a-c, was consistently upregulated by p53, which directly recognizes the promoters and activates the transcription of these miRNAs. These miRNAs function as powerful effectors to control p53-mediated cell cycle arrest and apoptosis

- It was identified another tumor suppressor miRNA, miR-200c, that is also controlled by p53.
- Through binding to the miR-200c promoter, p53 induces miR-200c expression and consequently attenuates EMT and reduces stem-cell-like population in breast cancer by targeting ZEB1 and BMI1, respectively

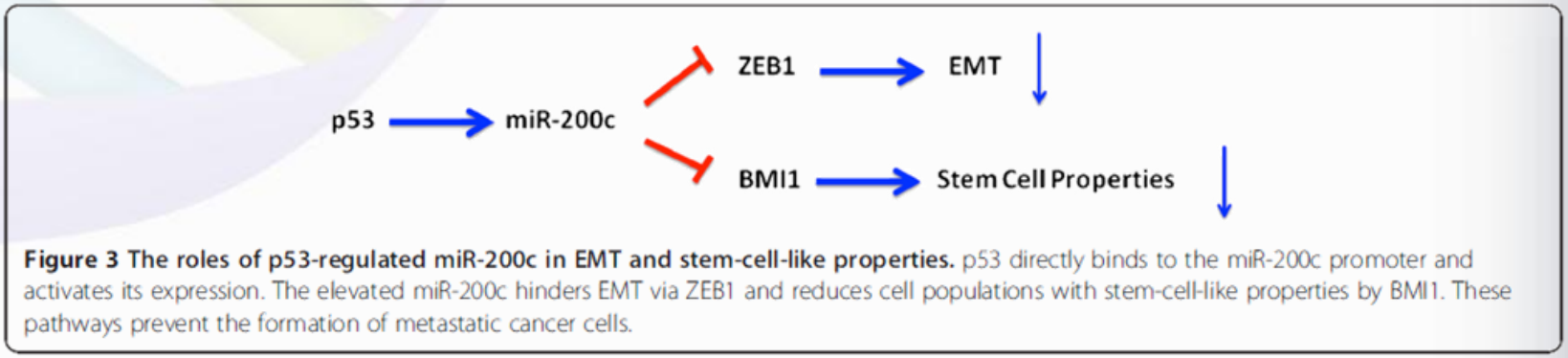


**Figure 3 The roles of p53-regulated miR-200c in EMT and stem-cell-like properties.** p53 directly binds to the miR-200c promoter and activates its expression. The elevated miR-200c hinders EMT via ZEB1 and reduces cell populations with stem-cell-like properties by BMI1. These pathways prevent the formation of metastatic cancer cells.

# 4. Abnormal maturation pathways

- After generation of primary miRNAs, a two-step Rnase-dependent
- maturation pathway is required to produce mature miRNAs.
- First, primary miRNAs (primiRNA) are processed by Drosha-containing complex to stem-loop pre-miRNAs, which are then further processed by the second RNase, Dicer, to short, double-strand duplexes.
- Eventually, one of the functional strands in the resulting duplexes is preserved, forming a functional complex with the RISC proteins, and acts as guiding strands for specific recognition. Currently, several RNA-binding proteins have been found to affect this canonical pathway with some that are involved in the regulation of cancer progression.

# Examples

- Lin-28 is the most studied RNA-binding protein being capable of regulating let-7 biogenesis. Overexpression of Lin-28 has been shown as an unfavorable prognostic marker in human cancers. Lin-28 modulates the structural alternation of pre-let-7g to inhibit Dicer dependent processing.
- The KH-type splicing regulatory protein (KSRP) was identified to enhance both Drosha- and Dicer-mediated miRNA processing through interaction with specific sequences in the loop region of a subset of pri-miRNAs. Knockdown of KSRP represses the expression of specific mature miRNAs, such as let-7a and miR-206, and consequently affects cell proliferation and differentiation.

# 5. miRNA-miRNA interaction

- After processing, mature miRNAs are produced as functional strands, loaded onto miRISC, and targeted to specific 3’UTRs, thereafter.

# Examples

- In addition to the binding between miRNA and 3’UTR of its target mRNA, recently identified a direct interaction between two individual miRNAs, miR-107 and let-7.
- It was identified the essential role of an internal loop within the miR-107::let-7 duplex, which provides important clues for further investigation on the underlying mechanism. MiR-107 mitigates the tumor suppressive effects of let-7, and thus facilitating cancer progression.